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Fluidextract of Ephedra: A Study of the Methods for its Preparation and Standardization

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FLUIDEXTRACT OF EPHEDRA:
A STUDY OF THE METHODS FOR ITS PREPARATION AND STANDARDIZATION

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This is to certify that, in accordance with the requirements of South Dakota State College for the Master of Science Degree, Guilford C. Gross has presented to this committee three bound copies of an acceptable thesis, done in the major field; and has satisfactorily passed a two-hour oral examination on the thesis, the major field, Pharmacology and Pharmacognosy, and the minor fields, Histology and Botany.

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HISTORICAL

Plants of the genus Ephedra have been used as empirical remedies in various parts of the world since ancient times. The Chinese herb, Ma Huang (Ma meaning astringent, Huang, yellow), (1) has been known and used by the Chinese for over 5000 years. The drug was tasted by the emperor, Shen Nung, some 5100 years ago and placed in the "medium class". (2) It appeared in the Chinese dispensatory, the "Pentsao Kang Mu" written in 1596 A. D. by Shi-Chang Li. (3). He stated it to be of value as a circulatory stimulant, diaphoretic, antipyretic, and sedative in cough.

Early Greek physicians used Ephedra plants as diuretics, and in the treatment of dysentery, cough, orthopnea and internal rupture. The Russians, in the 19th century prepared decoctions of the plant and administered them with butter and milk in the treatment of rheumatism, syphilis, and gout. The sap and candied fruits were used in respiratory disorders. In America, various species of Ephedra enjoyed considerable use in the treatment of venereal diseases and nephritis and as tonics and blood purifiers. The Mexicans used E. aspera and still do, in the treatment of pneumonia. (4)

Although, Ephedras have been used as empirical remedies since olden times, the beginning of their use in rational medicine has been comparatively recent. The active principle of Ma Huang was first isolated by G. Yamanashi in 1885 (5) but the substance he obtained was an impure product. Two years later, 1887, Nagai with the assistance of Y. Horid, succeeded in obtaining the active principle, the alkaloid ephed-

rine, in pure form.(6) Merck isolated the same principle in 1888. (7) Nagai applied the name ephedrine to the alkaloid.

In 1889, Ladenburg and Oelschlagel published their results on a second base, pseudoephedrine, prepared by Merck and obtained from a plant belonging to the same genus, Ephedra.(8) In 1902, Miller was able to isolate only pseudoephedrine and no ephedrine in the European variety. (9).

Miura, in 1887, studied the physiological properties of ephedrine and demonstrated the mydriatic effect of the alkaloid. (10) This led to the introduction of the principle into Western medicine as a new mydriatic. However, the toxic effects of the drug when used in large doses, led to its limited and brief use.

Asahina and Kubota in 1917 demonstrated the epinephrine-like action of the alkaloid. (11)

In 1923, Chen and Schmidt began the study of ephedrine and its properties. As a result of the work done by these and other recent investigators, this drug has now become of widespread use in occidental medicine, chiefly in the treatment of respiratory affections such as asthma, hay fever, bronchitis and whooping cough. (4)

Ephedrine was submitted to the Council of Pharmacy and Chemistry of the A. M. A. in 1926 and subsequently approved by it. (12) The alkaloid and two of its salts, the hydrochloride and sulfate, have been made official in the latest revision of the United States Pharmacopoeia, the U. S. P. XI. (13)

A BOTANICAL STUDY OF THE CHINA DRUG.

Plants of the genus Ephedra (Fam. Gnetaceae) are found widely distributed in nature. They have been found in the temperate and subtropical regions of Europe, Asia, and America. A great many species have been identified. Reports of 35, and even as high as 45 species have been given by various authors. (14, 15) Not all of these species, however, yield the alkaloid ephedrine; in fact, only a very few contain it in sufficient quantity to be of commercial importance.

The principle species of Ephedra, used as commercial sources of ephedrine are E. sinica, Stapf, E. equisetina Bunge, and Ephedra distachya Linne, all of which are found in Central Asia. E. equisetina Bunge and E. sinica are found in the mountainous regions of north China while E. distachya is collected in central China. (16)

Youngken, in his Textbook of Pharmaceutical Botany gives the following characteristics of the Gnetum family: Stems either unbranched and tuberous (Welwitschia) or branched and shrubby (Ephedra) or often climbing (Gnetum) and possessing open-collateral fibrovascular bundles. Resin canals are absent while true vessels (trachea) are present in the secondary xylem (distinction from conifers) Tracheids also occur in the xylem. Internodes and nodes are conspicuous in Ephedra and Gnetum leaves, often reduced to scale-like structures and arranged in opposite or verticillate fashion. Flowers nearly always dioecious and exhibiting a membraneous perianth of 2-4 bracts. Perianth of male flowers surrounding 2-8 stamens. Female flowers showing an erect ovule with 1-2 integuments and a bract-like perianth. Fruit in Ephedra, a reddish

somewhat globular, galbulus-like structure (syncarp); Seeds albuminous, the embryo with two cotyledons imbedded in the endosperm. (17)

Gathercoal and Wirth in 1936 state E. sinica to attain heights of 60-90 cm., with internodes varying from 40 to 60 mm. in length. (15) Youngken gives the height of E. sinica as 30 cm. and E. equisetina and E. distachya as 1-2 meters and 98 cm. respectively. Internodes vary from 11 to 23 mm. in equisetina to 60 mm. for sinica and distachya. (16)

The alkaloidal contents of various species of Ephedra vary considerably. Certain species have been found to contain no alkaloid at all whereas others yield greater than 1 per cent of total alkaloids. Feng and Read have assayed E. equisetina and report 1.73% of total alkaloids of which 85 to 90 per cent is ephedrine. (19) E. sinica yields a somewhat less amount of alkaloids, 1.32 per cent being reported by the same investigators. Feng and Read in 1938 also found that there is an increase in alkaloidal content from spring to fall. Their investigations of E. equisetina have revealed that the nodes contain less ephedrine than the internodes and that the roots, berries, seeds and woody stalks contain none at all. (19).

As yet, China is still the principal commercial source of ephedrine-yielding plants. However, experiments are now being conducted in this country to determine the possibility of cultivating the alkaloidal-bearing plant here. (20, 21)

THE PROPERTIES OF EPHEDRINE

The alkaloid, ephedrine, and two of its salts, the hydrochloride and sulfate are official in the U. S. P. XI. (13) Ephedrine alkaloid is a nitrogenous base designated as l-phenyl-2-methylaminopropanol-1. It exists as an unctuous, almost colorless solid, or as white to colorless crystals or granules. (13) It is soluble in water, in alcohol, in chloroform, in ether, and liquid petrolatum. (13) Aqueous solutions of the alkaloid are strongly alkaline to moistened, red litmus paper. The melting point varies from 34° to 40° C., depending upon the moisture content. It boils above 200° C. (Nagai, Merck). Aqueous solutions of ephedrine hydrochloride are levorotatory. The specific rotation at 25° C., using sodium light $(\alpha)_D$ is N.L.T. -33 and N.M.T. - 35.5 (U.S.P. XI) (13)

Ephedrine reacts with hydrochloric and sulfuric acids to form the corresponding salts. The hydrochloride occurs as fine, white, colorless crystals or powder, soluble one Gm. in three cc. of water, one Gm. in fourteen cc. of alcohol at 25° C. (13) It is insoluble in ether. (13) The sulfate is freely soluble in water and in hot alcohol; more difficultly soluble in cold alcohol. (13) The hydrochloride melts between 216° and 220° C., the sulfate between 243° and 247.5° C. (13).

A peculiar reaction of ephedrine is the formation of the hydrochloride when the alkaloid is shaken with chloroform. (22) Solutions of ephedrine or its salts react with few of the alkaloidal reagents.(4) With Mayers reagent, a turbidity or white precipitate is formed. (4)

Ephedrine gives characteristic micro-crystalline reactions when treated with Millon's reagent, gold chloride, plantinic chloride or Erdmann's reagent.(23) Salts of ephedrine have been shown to react with other reagents such as phosphomolybdic acid, phosphotungstic acid, Wagner's reagent, Erdmann's reagent, and with potassium dichromate and concentrated sulfuric acid. (1, 24) Ephedrine when treated with copper sulfate and sodium hydroxide gives a purple coloration which can be extracted with ether. This test is sensitive to one part in 400. (4) It was first demonstrated by Nagai in 1892. (25)

Ephedrine is isomeric with a second base, d-pseudoephedrine, which is also found in plants of the genus Ephedra. Pseudoephedrine ($C_6H_5CHOHCH(NHCH_3).CH_3$ melts at 117-118° C., is dextro-rotatory, and is readily soluble in ether and alcohol, but sparingly soluble in cold water. (24)

Ephedrine solutions are stable to light, air, heat, and age. Chen and Schmidt in 1930 report that a solution of ephedrine hydrochloride prepared and sealed in a sterile ampule on December 23, 1923, showed no change in appearance when opened on March 14, 1929, and produced the customary pressor response when injected into a pithed cat. (4).

THE ISOLATION AND ASSAY OF EPHEDRINE

The isolation of ephedrine from the crude drug, like that of other common alkaloids, follows two general steps: (a) extraction by means of a suitable solvent and (b) subsequent purification with the aid of immiscible solvents. A variety of solvents have been used in the extraction of the crude drug. Nagai, in his early work, used acidulated water, while others have used alcohol, mixtures of alcohol and water, ether, chloroform, and mixtures of the latter two. In those cases in which ether or chloroform are used, the drug is first alkalinised by mixing it with a suitable base. Chen, in 1925, used 80% alcohol in extracting the powdered drug. (1) The percolate thus obtained was concentrated, diluted with water, made alkaline with ammonia water and filtered. The alkaloid was extracted from the filtrate and precipitated by means of chloroform, and purified. Chen and Schmidt in 1930 used essentially the same procedure as above, but extracted the alkaloid from the crude drug with 60% alcohol. (4) The salt of the alkaloid was purified and repeatedly crystallised from absolute alcohol. Feng and Read, in their work, have employed a number of assay procedures, including extractions with acids (acetic and hydrochloric) and alkalinisation methods with subsequent extraction using chloroform. (26) They state that the low yield of ephedrine obtained by some workers was due to incomplete alkalinisation of the percolate before extraction with chloroform or ether, and emphasise the necessity of adding a large excess of ammonium hydroxide in order to liberate the alkaloids completely. (26) In a number of instances, pharmacopoeial methods for the assay of bul-

ladonna root and leaves have been employed (U. S. P. IX and X). Chen employed the U. S. P. IX method for the assay of belladonna root, in assaying Ephedra vulgaris, var. helvetica. (1) Schoetzow and Needham in 1926 used the same method on the same species, but obtained considerably greater quantities of alkaloid. (27) The collaborators of the Association of Official Agricultural Chemists have elaborated a method for the assay of Ephedra by which the alkaloids, after being liberated by a mixture of sodium carbonate and ammonia water, are taken up with a mixture of chloroform and ether. After purification by the acid shake-out process, the alkaloids are liberated by the dual alkali employed above, and extracted with ether. Titration is carried out using standard acid and alkali, with brom-thymol-blue as the indicator. (28) Rimer, in 1933, has suggested a similar method, but uses only ammonia water (stronger ammonia U. S.) in place of the dual alkali used above. (29) Williams in 1928 employed the U. S. P. X method for the assay of belladonna. (30) He states that the use of ether in extraction gives lower results than when ether-chloroform is used, and the final extraction of the aqueous solution with ether is much slower than with chloroform, although complete extraction may be obtained with 7 or 8 25 cc portions. He also states that maceration of the drug for two hours gives practically the same results as macerating for longer periods. (30)

Once the alkaloid has been obtained in a pure state, a chemical titration is readily carried out. An excess of standard acid is added, a residual titration being carried out using standard alkali. Brom-

thymol-blue has been used as the indicator, but in the second supplement to the U. S. P. XI, methyl red has been recommended to replace it in the assay of ephedrine and its salts. (31).

Biological assays for ephedrine and its salts have been studied by a number of investigators, but they do not appear to be as satisfactory as the chemical assay methods. Feng and Read in 1927 studied the pressor action of small doses of ephedrine on an amesthetized dog. (26.) Pittenger, in 1928, employed the same method on a number of dogs, but found that small doses (1 mg.) caused enough tachyphylaxis to render the method untrustworthy. (32) Githens, in 1933, describes a method based on the pressor action, which he states to be suitable. (33) However the ease and accuracy with which a chemical assay is carried out still renders this method the most desirable.

EXPERIMENTAL WORK

The work conducted here was concerned chiefly with extraction studies of Ephedra with the view in mind of obtaining a suitable procedure for the preparation of a fluidextract, and a method for its assay. In this connection, the following points were kept in mind:

1. To select a menstruum which would give a suitable extraction of the active principle.
2. To determine the optimum maceration period.
3. To establish a suitable process for conducting the extraction, and preparation of a fluidextract.
4. To select a suitable assay procedure for the completed fluidextract.
5. To study the stability of the fluidextracts under storage conditions.

THE CRUDE DRUG

All of the samples of crude drug used in the following experiments were collected from plants growing in the drug gardens at South Dakota State College. In all, four lots of drug were obtained. The green stems were collected, cleaned and dried at room temperature until needed, at which time they were ground to No. 40 powder, the moisture content determined, and kept in tightly closed bottles as used. The characteristics of the various samples are as follows:

Lot No. 1

Color of powder:	Light brown
Moisture (Oct. 24, 1939)	5.5%

This sample had been collected approximately one year before it was used.

Lot No. 2

Color of powder:	Light green
Moisture (Oct. 27, 1939)	6.8%

This sample was collected in the fall of 1939, during fruiting.

Lot No. 3

Color of powder:	Light green
Moisture (Nov. 18, 1939(	6.4%

Female plants collected in October, 1939.

Lot No. 4

Color of powder	Light green
Moisture (Nov. 15, 1939)	4.8%

Collected in the Fall of 1939.

ASSAY OF THE CRUDE DRUG

As a guide in further extractions, the four lots of Ephedra obtained above were assayed according to the method suggested by Hiner in 1939.(34) This method had been used here in assay work prior to this, and with satisfactory results. The procedure is as follows:

1. Use 10 Gm. samples in No. 60 powder, accurately weighed with allowance made for the moisture content. (11 Gm. samples of No. 40 powder were used in this work.)

2. Place in a suitable percolator, after first wetting the cotton pledget in the bottom with ether, saturate the drug with ether-chloroform menstruum (4:1) and, after allowing it to stand at least five minutes, add 10 cc. of stronger ammonia water. Mix this thoroughly with the drug, cover with an excess amount of menstruum and allow to macerate over night.

3. The next morning, pack the drug firmly and percolate at a moderate rate using ether-chloroform menstruum in the same proportion, 4:1. Continue the percolation until 300 to 400 cc. of percolate has been collected.

4. Transfer the percolate to a separatory funnel and completely extract the alkaloid by shaking with several portions of 2% sulfuric acid. Filter each portion as it is collected and then wash the complete acid extract with 3, 40 cc. portions of ether to remove traces of chloroform which may have been carried through.

5. Add a portion of ether to the acid solution, make strongly alkaline with a decided excess of stronger ammonia water, and complete-

ly extract the freed alkaloid with several successive portions of ether. A Folkin-Mathins continuous extraction apparatus can be used to good advantage in this step. The care of the assay from this point to completion is precisely the same as that recommended by the U. S. P. XI for the assay of ephedrine salts. Each cc. of N/10 hydrochloric acid corresponds to .01651 Gms. of ephedrine alkaloid.

Below are the results obtained for the above four lots of Ephedra:

Lot No. 1	.89 per cent ephedrine.
Lot No. 2	.31 per cent ephedrine.
Lot No. 3	.63 per cent ephedrine.
Lot No. 4	.78 per cent ephedrine.

EXTRACTION STUDIES

Extraction studies were conducted on the above lots of Ephedra. The following menstrua were used:

1. Alcohol.
2. Alcohol, 9 volumes, water 1 volume (no assay).
3. Alcohol, 7 volumes, water 1 volume (no assay).
4. Alcohol, 4 volumes, water 1 volume.
5. Alcohol, 3 volumes, water 1 volume.
6. Alcohol, 2 volumes, water 1 volume.
7. Alcohol, 1 volume, water 1 volume.
8. Alcohol, 1 volume, water 2 volumes (no assay).
9. (a) Diluted alcohol 48.5 cc., hydrochloric acid 1.5 cc.
   (b) Diluted alcohol.

Data for the extractions are given in the following tables.

No. 1

Extractions Using Alcohol as the Menstruum.

No. Extraction	1	2
Drug	Lot 1	Lot 1
Weight	60.00 Gm.	25.00 Gm.
Percolator	Cylindrical	Alkaloidal
Maceration Period	48 Hr.	24 Hr.
Volume Percolate	300 cc.	100 cc.
Weight Marc*	54.50 Gm.	23.50 Gm.
Loss in Weight (including moisture)	5.50 Gm.	1.50 Gm.
Per Cent Loss	9.10 %	10.00 %
Assay		
Total Alkaloid	.101 Gm.	———
Per Cent	.168 %	———
Method	1 (a)	———

* Marc dried at 85° C., overnight, cooled and weighed.

No. 2

Extractions Using Alcohol-Water (4:1) as the Menstruum

No. Extractions	1	2	3
Drug	Lot 1	Lot 1	Lot 4
Weight	60.00 Gm.	25.00 Gm.	25.00 Gm.
Percolator	Cylindrical	Alkaloidal	Alkaloidal
Maceration Period	48 Hr.	24 Hr.	24 Hr.
Volume Percolate	300 cc.	100 cc.	100 cc.
Weight Marc*	43.20 Gm.	18.00 Gm.	19.80 Gm.
Loss in Weight (including moisture)	16.80 Gm.	7.00 Gm.	5.20 Gm.
Per Cent Loss	28.00 %	28.00 %	20.80 %
Assay			
Total Alkaloid	.558 Gm.	———	.165 Gm.
Per Cent	.93 %	———	.663 %
Method	1 (a)	———	1 (b)

* Marc dried 85° C., overnight, cooled and weighed.

No. 1

Extractions Using Alcohol-Water (4:1) as the Menstruum and Varying the Period of Maceration.

No. Extraction	1	2	3	4
Drug	Lot 4	Lot 4	Lot 4	Lot 4
Weight	25.00 Gm.	25.00 Gm.	25.00 Gm.	25.00 Gm.
Percolator	Alkaloid.	Alkaloid.	Alkaloid.	Alkaloid.
Maceration Period	12. Hr.	24 Hr.	48 Hr.	72 Hr.
Volume Percolate	100. cc.	100 cc.	100. cc.	100 cc.
Weight Marc*	19.50 Gm.	19.60 Gm.	20.20 Gm.	19.70 Gm.
Loss in Weight (includ. moisture)	5.50 Gm.	5.40 Gm.	4.80 Gm.	5.30 Gm.
Per Cent Loss	22.00 %	20.60 %	19.20%	21.20 %
Assay				
Total Alkaloid	.151 Gm.	.168 Gm.	.165 Gm.	.157 Gm.
Per Cent	.60%	.67%	.66%	.63%
Method	1 (b)	1 (b)	1 (b)	1 (b)

* Marc dried 85° C., overnight, cooled and weighed.

No. 4

Extractions Using Alcohol-Water (3:1) as the Menstruum

No. Extraction	1	2	3
Drug	Lot 1	Lot 1	Lot 4
Weight	60.00 Gm.	25.00 Gm.	25.00 Gm.
Percolator	Cylindrical	Alkaloid.	Alkaloid.
Maceration Period	48 Hr.	24 Hr.	24 Hr.
Volume Percolate	300 cc.	100 cc.	100 cc.
Weight Marc.*	43.00 Gm.	17.90 Gm.	19.40 Gm.
Loss in Weight (including moisture)	17.00 Gm.	7.10 Gm.	5.60 Gm.
Per Cent Loss	28.30%	28.40 %	22.40 %
Assay			
Total alkaloid	.493 Gm.	——	.198 Gm.
Per Cent	.82 %	——	.63 %
Method	1 (a)	——	1 (b)

* Marc dried 85° C., overnight, cooled and weighed.

No. 5

Extractions Using Alcohol-Water (2:1) as the Menstruum.

No. Extraction	1	2	3	4
Drug	Lot 1	Lot 1	Lot 4	———
Weight	60. Gm.	25. Gm.	25. Gm.	50. Gm.
Percolator	Cylindrical	Alkaloid.	Alkaloid.	Cylindrical.
Maceration Period	48 Hrs.	24 Hrs.	24 Hrs.	24 Hrs.
Volume Percolate	300 cc.	100 cc.	100 cc.	100 cc.
Weight Marc*	43. Gm.	17.7 Gm.	18.8 Gm.	40.5 Gm.
Loss in Weight (includ. moisture)	17. Gm.	7.3 Gm.	6.2 Gm.	9.5 Gm.
Per Cent Loss	28.3%	29.2%	24.8%	19.0%
Assay				
Total alkaloid	.493 Gm.	———	.114 Gm.	.184 Gm.
Per Cent	.82%	———	.45%	.36%
Method	1 (a)	———	1 (b)	3

* Marc dried 65° C., overnight, cooled and weighed.

No. 6

Extractions using Diluted Alcohol as the Menstruum

No. Extraction	1	2	3	4	5
Drug	Lot 1	Lot 1	Lot 4	———	———
Weight	60. Gm.	25 Gm.	25 Gm.	50 Gm.	50 Gm.
Percolator	Cylindr.	Alkaloid.	Alkaloid.	Cylindr.	Cylindr.
Mac. Period.	48 Hrs.	25 Hrs.	24 hrs.	24 Hrs.	48 Hrs.
Vol. Perc.	300 cc.	100 cc.	100 cc.	100 cc.	50 cc.
Weight Mare*	42.5 Gm.	17.5 Gm.	18.9 Gm.	38.2 Gm.	41.0 Gm.
Loss in Wt. (inc. moisture)	17.5 Gm.	7.5 Gm.	6.1 Gm.	11.8 Gm.	9.0 Gm.
Per Cent Loss	29.1%	30.0%	24.4%	23.6%	18.0%
Assay					
Total alkaloid	.356 Gm.	———	———	.145 Gm.	.193/100 .096/50cc.
Per Cent	.5%	———	———	.29%	.19%
Method	1 (a)	———	———	3	3

* Mare dried 65° C., overnight, cooled, and weighed.

No. 7

Extractions Using Acid Menstruum.

Menstruum: (a) Diluted Alcohol 48.5 cc.
HCl 1.5 cc.

(b) Diluted Alcohol.

No. Extraction	1
Drug	———
Weight	50.00 Gm.
Percolator	Cylindrical
Maceration Period	24 Hours.
Volume Percolate	105 cc.
Assay	
Total Alkaloid/100 cc.	.226 Gm.
Per Cent Alkaloid/105 cc.	.475
Method	3

No. 8

Extractions Using Various Other Alcoholic Menstrua

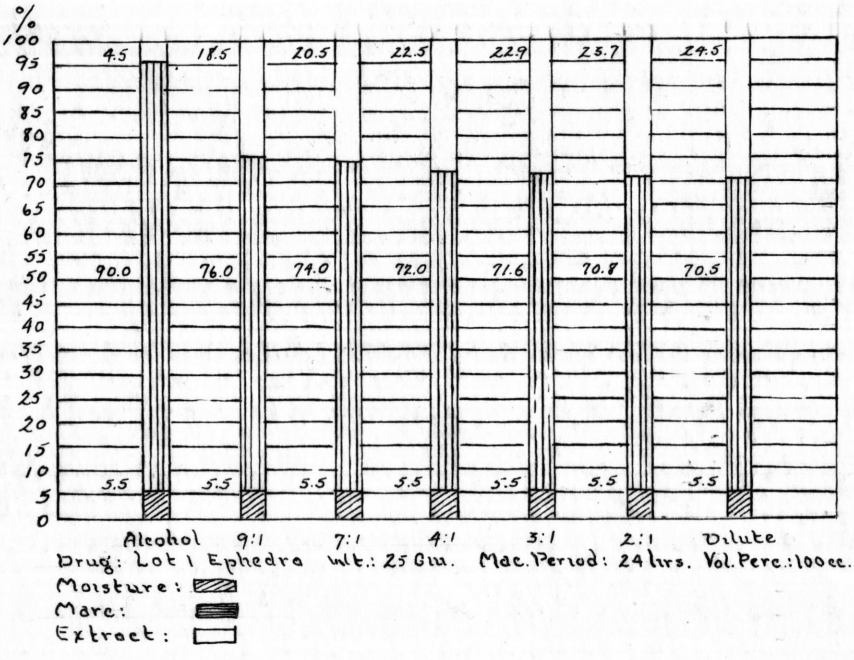
(No assays were obtained for these.)

No. Extraction	1	2	3
Drug	Lot 1	Lot 1	Lot 4
Menstruum	Alc. 9:1	Alc. 7:1	Alc. 1:2
Ext. Period	24 hrs.	24 hrs.	24 hrs.
Weight	25 Gm.	25 Gm.	25.Gm.
Vol. Percolate	100 cc.	100 cc.	100 cc.
Weight Marc*	19 Gm.	18.5 Gm.	18.9 Gm.
Loss Weight (including moisture)	6 Gm.	6.5 Gm.	6.1 Gm.
Per Cent Loss	24 %	26 %	24.4%

* Marc dried 85° C., overnight, cooled and weighed.

OBSERVATIONS AND CONCLUSIONS.

It appears from the work conducted here, that the higher alcoholic menstrua extract a smaller proportion of the crude drug than do the lower alcoholic menstrua. There was a progressive increase in the quantity of the extracted material, as the percentage of alcohol in the menstrua decreased. The graph below shows the comparative quantities of material extracted by various menstrua



Ephedrine appears to be readily extracted by various alcoholic menstrua. Best extractions were obtained using alcohol-water in the ratios 4:1, 3:1, and 2:1. Of these, alcohol-water 4:1, gave somewhat better results than the other two.

There did not appear to be any correlation between the length of the maceration period and the quantity of alkaloid extracted. Thus, in using alcohol-water 4:1, a 24-hour maceration period gave as high results as long periods (48 and 72 hours). Macerating the drug 12 hours, however, gave slightly lower results than did the 24-hour period.

From the results obtained, it would appear that any of the three menstrua mentioned above would be suitable in the preparation of a fluidextract.

PREPARATION OF FLUIDEXTRACTS

A number of fluidextracts were prepared using the menstrua which had been used in the foregoing extractions. Two general types of fluidextracts were prepared, namely, (a) so-called resin-precipitated fluidextracts and (b) those made by an official type process of the U. S. P. XI. (Type Process A and C).

Resin-Precipitated Fluidextracts

It was brought to attention that alcoholic extracts of Ephedra sent in a resin-like principle which may be an undesirable constituent of a fluidextract. The fact that an actual resinous principle exists in plants of this genus is doubtful, in fact, Youngken (16) states that resin canals are absent in plants of the Gnetum family. No mention was made in the various books and journals available of the actual occurrence of a resin. The following work which was carried out in preparing fluidextracts of this type did not indicate that resinous material was extracted, but rather, the chief material precipitated in the case of the higher alcoholic menstrua was chlorophyll.

The following menstrua were used in preparing fluidextracts of this type: (1) alcohol, (2) alcohol-water, 4:1, (3) alcohol-water, 3:1, (4) Alcohol-water, 2:1, (5) diluted alcohol. Reference was here made to a procedure used by Eli Lilly and Company (35) and to the official process for the preparation of Fluidextract of Ipecac, U. S. P. XI.

Fluidextract No. 1

Menstruum: Alcohol

Drug: Lot 1 Ephedra

Procedure: 60 Gm. of drug in No. 40 powder was mixed with a sufficient quantity of the menstruum to render damp, then placed in a cylindrical percolator of suitable capacity, and, after adding a sufficient quantity of the menstruum to saturate and leave a stratum above the drug, was allowed to macerate 48 hours. Percolation was allowed to proceed slowly until 300 cc. of percolate had been collected. The percolate was deep green, showing the extraction of chlorophyll from the drug. The percolate was evaporated spontaneously (at about 25° C.) to a volume of 100 cc. and 200 cc. of distilled water was added. The mixture thus obtained was allowed to stand 24 hours and then it was filtered. The filtrate was light orange in color. The filtrate was assayed and the remaining portion evaporated to a soft extract, spontaneously, and finally diluted with alcohol-water in the ration of 1:4 to 46.8 cc. to give a fluidextract assaying .2%.

The filtrate was evaporated to a soft extract rather than just to the required volume, in order that the alcoholic content of the finished product could be readily adjusted.

The precipitated material, after drying at room temperature for two days weighed approximately 1.5 Gm. and was greenish-black in color. It appeared to be chiefly chlorophyll.

Fluidextract No. 2

Menstruum: Alcohol, 4 volumes, water, 1 volume.

Drug: Lot 1 Ephedra

Procedure: This fluidextract was prepared in the same manner as that described above. After filtration, the filtrate was assayed, evaporated spontaneously to a soft extract and then diluted with alcohol-water in the ratio of 1:4 to 64.8 cc. to give a fluidextract assaying .6%.

The precipitated material obtained above was greenish-black in color and weighed approximately 4 Gm. after drying at room temperature.

Fluidextract No. 1

Menstruum: Alcohol, 3 volumes, water, 1 volume

Drug: Lot 1 Ephedra.

Procedure: This fluidextract was prepared in the same manner as above with the following exceptions: After the addition of the water to the alcoholic percolate, the mixture was allowed to stand 48 hours before filtering. The filtrate was assayed, evaporated spontaneously to a soft extract and then diluted to 90.6 cc. with alcohol-water in the ratio 1:4 to give a fluidextract assaying .5%.

The precipitated material was greenish-black and weighed approximately 6.5 Gm.

Fluidextract No. 4

Menstruum: Alcohol 2 volumes, water, 1 volume.

Drug: Lot 1 Ephedra.

Procedure: The percolate obtained as above was concentrated to a thin syrup and diluted with alcohol-water, 2:1 to 100 cc. Precipitation was carried out by the addition of 125 cc. of distilled water. After standing 48 hours, the mixture was filtered, assayed, evaporated spontaneously to a soft extract and then diluted to 90.8 cc. with alcohol-water 1:4 to give a fluidextract assaying .5%.

The precipitated material was greenish-black and weighed approximately 7 Gm.

Fluidextract No. 5

Menstruum: Diluted Alcohol

Drug: Lot 1 Ephedra

Procedure: The percolate, obtained as above, was evaporated to a thin syrup, diluted to 100 cc. with diluted alcohol, and 100 cc. of water added. The mixture was allowed to stand 48 hours and was then filtered. The filtrate was assayed, concentrated, and diluted with alcohol-water 1:4 to 62 cc. to give a fluidextract assaying .3%.

The precipitated material was greenish-black in color and weighed approximately 7.4 Gm.

In assaying the fluidextracts prepared above, the assay figures were not regarded as an index for the relative completeness of extrac-

tion, inasmuch as some of the alkaloid may have been lost during the process of precipitation. Thus, those figures which are given under extractions, pages 16-20 (in each case it is the first extraction) represent the quantities of alkaloid remaining in the percolate after precipitation. Evaporation temperatures were kept low, to prevent any loss of alkaloid by this means.

Fluidextract No. 6

None of the fluidextracts prepared above proved to be very stable. In view of this fact, a sixth fluidextract was prepared, in which the alcoholic concentration of the finished product was increased. The process employed is given below, with the exception that the fluidextract was adjusted so that 1 cc. = 1 Gm. of drug, the preparation preparation not being assayed. The most suitable menstruum appeared to be alcohol-water in the ratio of 4:1, and a maceration period of 24 hours.

Procedure: Mix 100 Gm. of drug, in No. 40 powder with a sufficient quantity of the menstruum to render it evenly and distinctly damp. Place the drug in a percolator of suitable capacity and after a preliminary maceration period of 15 minutes, pack and add a sufficient quantity of the menstruum to saturate and leave a stratum above the drug. When the menstruum is ready to drip from the lower orifice, close and allow to macerate 24 hours. Exhaust the drug using the prescribed menstruum. Now concentrate the percolate at a temperature not exceeding 60° C. to a volume of 100 cc. Add 300 cc. of distilled

water, let stand overnight, and filter. Now concentrate the filtrate to a volume of 61.5 cc. at a temperature not exceeding 60° C., and add 23.5 cc. of alcohol. Assay a portion of the percolate and dilute the remainder to conform to standard using a mixture of 23.5 cc. of alcohol and 75.5 cc. of distilled water. Each 100 cc. of finished fluidextract should contain N.L.T. .75 Gm. and N.M.T. .85 Gm. of ephedrine alkaloid*.

The fluidextract prepared in this manner has been kept 70 days and the amount of precipitation is slight. It would have been advisable at the time of precipitation to use acidulated water, since this would prevent volatilization of the alkaloid (due to formation of salt) during further evaporation. Evaporating at the temperature above would probably result in loss of the active principle.

Other Resin-Precipitated Fluidextracts

Several other resin-precipitated fluidextracts were prepared according to the general method given for fluidextract No. 6. These were adjusted so that 1 cc = 1 Gm., but the alcoholic concentrations were varied as follows: 20%, 26%, 31%, and 41%. The preparations were stored and observed at a later date.

Fluidextracts Prepared by an Official Type Process

A number of fluidextracts were prepared using methods given in the U. S. P. XI. The following menstrua were employed: Alcohol-water

* This standard was used so that the finished fluidextract would conform to the standard for the crude drug which is to assay .8%. (Tentative Monograph, Hines)

4:1, alcohol-water 2:1, and diluted alcohol. Type Process A was employed in the case of each of the above menstrua. One fluidextract was made using type process C, with alcohol-water in the ratio of 4:1 as the menstruum. The fluidextracts were adjusted so that 1cc. = 1 Gm. and were stored, to be observed at later dates.

STORAGE

The fluidextracts prepared above were stored for periods ranging from 70 days to 142 days. With few exceptions, they were all kept under identical conditions of storage, that is, in small tightly stoppered glass bottles, in the absence of direct sunlight and at a temperature of 20° to 25° C. The stabilities of the various preparations are given below.

Resin-Precipitated Fluidextracts

Fluidextract	Period of Storage	Result
1	142 days	heavy ppt.
2	140 days	heavy, gummy ppt.
3	139 days	heavy, gummy ppt.
4	139 days	gummy ppt.
5	139 days	slight ppt.
6	70 days	negligible ppt.
7 (p. 30, Alc. 20%)	72 days	slight ppt.
8 (p. 30, Alc. 26%)	72 days	negligible ppt.
9 (p. 30, Alc. 31%)	72 days	negligible ppt.
10 (p. 30, Alc. 43%)	72 days	negligible ppt.

Other Fluidextracts

11 (Type A, Alc. 45%)	78 days	slight, gummy ppt.
12 (Type A, Alc. 45%)	78 days	slight, gummy ppt.
13 (Type A, Alc. 45%)	78 days	slight, gummy ppt.
14 (Type C, Alc. 45%)	75 days	no precipitate.
15 (Type C, Alc. 45%)	75 days	no precipitate.
16 (Type C, Alc. 45%)	75 days	no precipitate.

Fluidextracts prepared by Type Process A, using alcohol-water 2:1 and diluted alcohol as the menstrum, were not stored for any appreciable period.

Fluidextract 11 was kept at 40° F. for two days, at the end of which time, a heavy precipitate was noted, but which redissolved on warming and shaking.

Two of the fluidextracts (12 and 13) were kept in sunlight. However, precipitation did not appear to be any greater than in the case of those kept in the absence of sunlight.

ASSAY PROCEDURES

The assay procedures employed here were based on the separation and purification of the alkaloid by means of immiscible solvents and the subsequent titration of the alkaloid using a standard acid and alkali. Several procedures were employed in the purification of the alkaloid, and, in general, are as follows:

(1) Direct extraction of the alkaloid with ether, from the alkalinised fluidextract.

(2) Precipitation with dilute acid, with subsequent alkalinisation, filtration, and extraction of the alkaloid with ether.

(3) Precipitation with strong alkali, with subsequent filtration and extraction of the alkaloid with ether.

Ether was used as the immiscible solvent in preference to chloroform, because in many cases, the latter solvent caused troublesome emulsions.

In all cases, titrations were carried out using N/50 sodium hydroxide as the standard alkali and N/50 hydrochloric acid as the standard acid. Methyl red was used as the indicator.

The assay methods employed are given as follows:

(1a) The following procedure was used for assaying the so-called resin precipitated fluidextracts (1 - 5). (36) It appeared to be a quite satisfactory procedure for all fluidextracts thus prepared except in the case where diluted alcohol was used as the menstruum, in which case some emulsification was encountered and the results obtained were

doubtful. The method used is as follows:

Transfer 20 cc. of the sample, accurately measured to an automatic extraction apparatus (Palkin-Gathins). Add 30 cc. of distilled water, 10 cc. of 2% sulfuric acid and extract with ether for two hours. Disconnect the receiving flask, discard the acid-ether extraction and replace it with clean ether. Now add an excess of stronger ammonia water (about 10 cc.) and extract the liberated alkaloid with ether during a period of four hours. Disconnect the receiving flask from the extractor and cautiously evaporate the ether on a water bath to a volume of about 5 cc. Cool the residue and add 20 cc. of N/50 hydrochloric acid, then continue to warm on the water bath until all of the ether is expelled. Now cool and titrate the excess of acid with N/50 sodium hydroxide, using methyl red as the indicator. Each cc. of fiftieth normal acid is equivalent to .003302 Gm. of ephedrine alkaloid.

The figures below represent the quantities of ephedrine alkaloid found in the above filtrates, when assayed by this method.

Fluidextract No. 1	.101 Gm. ephedrine
Fluidextract No. 2	.358 Gm. ephedrine
Fluidextract No. 3	.492 Gm. ephedrine
Fluidextract No. 4	.492 Gm. ephedrine
Fluidextract No. 5	.356 Gm. ephedrine.

While this method was quite satisfactory for assaying the above fluidextracts, it was of no value in assaying percolates in which the quantity of chlorophyll was high. In such cases, the two hour extraction with ether of the acid solution, did not give complete extraction

the original flask and rinse it thoroughly with 10 cc. of distilled water, made slightly alkaline with a few drops of strongest ammonia water. Filter this mixture, using the same filter paper as before and collecting the filtrate in the separatory funnel. Rinse the flask with a small portion of distilled water, and filter this into the separatory funnel. Extract the combined filtrates, with repeated portions of ether. Concentrate the ether extract on a water bath (cautiously) to about 5 cc. Add 15 cc. of alcohol, neutralised to methyl red T.S. and warm to expel the remaining ether. Now, add an excess of N/50 hydrochloric acid. Titrate the excess acid using N/50 sodium hydroxide, with methyl red T. S. as the indicator. Each cc. of N/50 acid used is equivalent to .003308 Gm. of ephedrine alkaloid.

The figures given below indicate the accuracy of this method.

1. Assay of percolate obtained under Extraction 4, page 19.
   - (a) .009 Gm. ephedrine alkaloid per 10 cc.
   - (b) .010 Gm. ephedrine alkaloid per 10 cc.
   - (c) .010 Gm. ephedrine alkaloid per 10 cc.

2. Assay of percolate obtained under Extraction 4, page 20.
   - (a) .012 Gm. ephedrine alkaloid per 10 cc.
   - (b) .007 Gm. ephedrine alkaloid per 10 cc.
   - (c) .014 Gm. ephedrine alkaloid per 10 cc.
   - (d) .014 Gm. ephedrine alkaloid per 10 cc.

3. Assay of percolate obtained under Extraction 5, page 20.
   - (a) .016 Gm. ephedrine alkaloid per 10 cc.
   - (b) .019 Gm. ephedrine alkaloid per 10 cc.

of this constituent and as a result, there was chlorophyll contamination in the alkaline-ether extraction. To eliminate this source of error, the acidified percolate was extracted with several portions of ether, in a separatory funnel and then the procedure was carried out as before (method 1b). The danger of emulsification, especially in assaying percolates of low alcoholic menstrua, was still present. The above methods proved to be far from satisfactory in assaying all types of fluidextracts.

(2) An attempt was made to assay fluidextracts prepared with diluted alcohol and alcohol-water 2:1, using a similar method to that given under Fluidextract of Belladonna Root, U. S. P. XI. The method was unsatisfactory inasmuch as the precipitate formed by the addition of acid to the sample was so fine that it was difficult to filter and because the subsequent addition of ammonium hydroxide to the filtrate caused the formation of a heavy precipitate which interfered with the extraction with ether.

(3) A third procedure was employed, in which ammonium hydroxide was added to the sample, the mixture filtered and the filtrate extracted with ether. The method was applied to extractions obtained with diluted alcohol (Extractions 4 and 5, page 20), diluted alcohol and acid (Extraction 1, page 21), and alcohol-water 2:1 (Extraction 4, page 19). The method employed was as follows:

Transfer 10 cc. of the fluidextract, accurately measured to a suitable flask, and add 10 cc. of distilled water and 5 cc. of stronger ammonia water. Now mix thoroughly, and filter the mixture, collecting the filtrate in a separatory funnel. Transfer the precipitate to

4. Assay of Percolate obtained under Extraction 1, page 21

(a) .019 Gm. ephedrine alkaloid per 10 cc.

(b) .020 Gm. ephedrine alkaloid per 10 cc.

(c) .024 Gm. ephedrine alkaloid per 10 cc.

(d) .023 Gm. ephedrine alkaloid per 10 cc.

(e) .025 Gm. ephedrine alkaloid per 10 cc.

The assay results obtained above were not too consistent. In the first case, there was a slight amount of chlorophyll contamination due to the fact that not all of the alcohol was removed before filtration. The assay figures in this case showed a range of from .005 Gm./10 cc. to 0.10 Gm./10 cc. with a difference of .01 Gm. Somewhat better results were shown in the other three assays, although occasionally, one result would vary considerably from the others. Thus, in the second series, two assays showed .024 Gm. of alkaloid per 10 cc. It is probable that the loss of alkaloid occurs in the process of precipitation and filtration; that is, that the alkaloid is not completely removed from the precipitate.

From the results shown it appears that the above method, with some modifications, might prove to be satisfactory. Two alterations in the above method come to mind: (1) It appears that the mixture obtained prior to filtration should be concentrated to remove any alcohol which is present and (2) it is necessary to obtain a more complete extraction of the alkaloid from the precipitated material.

The work on assay procedures is not entirely satisfactory and it is necessary that this work be continued, before a suitable procedure

may be suggested. The last method employed seems to be the most promising and work will be conducted along this method.

SUMMARY

1. A brief review of the literature was made.
2. Four lots of Ephedra sinica were collected from the drug gardens at South Dakota State College, their moisture contents determined and the samples assayed.
3. Extraction studies were conducted using various alcoholic menstrua. Of those menstrua employed, alcohol and water in the ratio of 4:1 gave the most complete extraction of ephedrine.
4. A study of the maceration period was made. It was found that a maceration period of 24 hours is sufficient to give a satisfactory extraction of the alkaloid.
5. Two general types of Fluidextracts were prepared, using various alcoholic menstrua: (1) the so-called resin-precipitated fluidextracts and (2) those prepared by official type processes of the U. S. P. XI (Type Processes A and C). As far as was determined those prepared by the former method were not superior to those prepared by the Type Processes.
6. The stabilities of the various fluidextracts were studied.
7. A study of assay procedures was made. Of the procedures employed, method 3, page 20 appeared to be the most promising, although it was not entirely satisfactory.

FLUIDEXTRACTUM EPHEDRAE N. F. (Tentative)

Fluidextract of Ephedra

Fldext. Ephed.

Fluidextract of Ma Huang.

Fluidextract of Ephedra yields, from each 100 cc. not less than .75 Gms. and not more than .85 Gms. of the alkaloids of Ephedra.

Prepare the Fluidextract from Ephedra in moderately coarse powder, by Process A* (see page 154). Use a mixture of 4 volumes of alcohol and 1 volume of water as the menstruum, macerate the drug 24 hours, and percolate at a moderate rate.

Assay: Transfer 10 cc of the fluidextract accurately measured, to an evaporating dish, and add 10 cc of distilled water and 5 cc of stronger ammonia water. Cautiously evaporate the mixture on a water bath to a volume of about 10 cc. and add 10 cc. of distilled water and filter, collecting the filtrate in a separatory funnel. Transfer the residue on the filter paper to the evaporating dish, mix thoroughly with a portion of distilled water made alkaline with stronger ammonia water, and filter through the same filter paper into the separatory funnel. Finally rinse the evaporating dish with a third portion of distilled water and pass this through the filter paper. Extract the combined filtrates with successive portions of ether, testing for complete extraction with Mayer's Reagent. Cautiously evaporate the ether extract on a water bath to a volume of about 5 cc. and add 10 cc. of alcohol made neutral to Methyl red T.S., with fiftieth-normal sodium hydroxide. Add an excess of N/50 hydrochloric acid. Titrate the excess of acid with N/50 sodium hyroxide using Methyl red T. S. as the indicator. Each cc. of N/50 hydrochloric

acid used is equivalent to .003302 Gm. of ephedrine.

(This procedure is only tentative and is subject to confirmation.)

Average dose: .03 cc. (½ minim).

* Type Process C (see page 155) as alternative.

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